Addendum #1 to Data Evaluation Record

MRID: 50444502
PC Code: 005210
OPPTS Guideline: Non-Guideline Semi-Field and Residue Study

Date: July 15, 2019

DER Study Title: GF-2626 (Sulfoxaflor): Brood Development of the Honey Bee (*Apis mellifera* L.) in a Colony Feeding Test in Germany 2016

Additional Reference: USEPA. 2019. Sulfoxaflor: Ecological Risk Assessment for Section 3 Registration for Various Proposed New Uses. (PC Code 005210; DP Barcode 449891). U.S. Environmental Protection Agency, Office of Pesticide Programs, Environmental Fate and Effects Division. Dated July 10, 2019.

Changes Made: The incorrect PC code is listed on the document (005211). The PC Code should be 005210.

Study Classification: No change

Revised by:
Meghann Niesen, Biologist
OPP/EFED/ERB-5

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Date: 2019.07.15 13:00:41 -04'00'

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2019.07.15
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DATA EVALUATION RECORD
HONEYBEES FIELD TEST
Apis mellifera
Colony Feeding and Field Trial Residue Study

1. **CHEMICAL:** Sulfoxaflor — PC Code No.: 005211

2. **TEST MATERIAL: GF-2626 (ai: Sulfoxaflor)** — Purity: 125 g ai/L; 11.8% w/w (analyzed)

3. **CITATION**

Author: Szczesniak, B.

Title: GF-2626 (Sulfoxaflor): Brood Development of the Honey Bee (*Apis mellifera* L.) in a Colony Feeding Test in Germany 2016

Study Completion Date: July 24, 2017

Laboratory: Eurofins Agroscience Services EcoChem GmbH / Eurofins Agroscience Services Ecotox GmbH
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Sponsor: Dow Agrosciences Ltd.
3B Park Square
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Oxfordshire, OX14 4RN
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Laboratory Report ID: S16-01455

DP Barcode: 445191

MRID No.: 50444502

4. **REVIEWED BY:** Adrian Graff, Environmental Scientist, CDM/CSS-Dynamac JV

Signature: Adrian T. Graff **Date:** 4/11/2018

APPROVED BY: Moncie V. Wright, Environmental Scientist, CDM/CSS-Dynamac JV

Signature: Moncie V Wright **Date:** 5/11/2018

5. **APPROVED BY:** Keith Sappington, Senior Science Advisor, OPP/EFED/ERB5

Signature: Digitally signed by Keith Sappington Date: 2019.07.09 22:20:10 -04'00' **Date:** 7/9/2019

Secondary Reviewer: Thomas Steeger, Senior Science Advisor, OPP/EFED/ERB4 Digitally signed by Keith Sappington Date: 2019.07.10 09:13:10 -04'00'

6. **DISCLAIMER:** *This Data Evaluation Record may have been altered by the Environmental Fate and Effects Division subsequent to signing by CDM/CSS-Dynamac JV personnel. The CDM/CSS-Dynamac Joint Venture role does not include establishing Agency policies.*

7. **STUDY PARAMETERS**

Test Species: Honeybees (*Apis mellifera* L.)
Age of Test Organism at Test Initiation: Healthy and queen-right colonies contained one queen and one body with 10 combs each, 7670 to 9945 adult bees/colony, 5-10 brood combs with all brood stages, and 3-10 honey and pollen combs
Test Duration: 10-day exposure period with additional monitoring in the fall and following spring after overwintering (302DAF, DAF = days after feeding).

8. **CONCLUSIONS:** The effects of the sulfoxaflor formulated end-use product Closer (GF 2626; 12% a.i.) was evaluated in a honey bee (*Apis mellifera*) colony feeding study. Colonies were provided 200 mL of diets containing untreated 50% sucrose (control) or sucrose diets at 0.02, 0.1, 0.5, 2, or 4 mg ai/kg each day for 10 consecutive days. Six colonies were used in each treatment group; five of the colonies were used for biological measurements and one colony was used for monitoring residues. Two additional treatments (each with 3 colonies) received diets containing reference toxicants dimethoate or fenoxycarb). Study colonies ranged in size from 7849 to 9,945 adult bees. Following the 10-day exposure phase of the study, the colonies were monitored through the spring of the following year (*i.e.*, overwintering). Colony condition assessments (CCAs were conducted twice before the exposure phase, 12 times after the exposure phase and once after overwintering. Bee mortality was evaluated daily from 4 days before feeding (4 DFB) to 44 days after feeding (44 DAF). Two complete honey bee brood (egg → larvae → pupae) cycles were evaluated: brood cycle 1 from 1 DBF to 20 DAF and brood cycle 2 from 15 DAF to 43 DAF during which time brood development indices were measured.

The lowest observed adverse effect concentration (LOAEC) in this study is based on sustained and statistically significant ($p<0.05$) differences (reductions) relative to controls in the number of adults bees and brood; increased worker and larval mortality during Weeks 1 and 2 after the 10-day exposure period; reductions in colony weight; and, reduced honey stores after overwintering in colonies exposed to sulfoxaflor at nominal dietary concentrations of 2 mg ai/kg (measured 1.85 mg ai/kg). The no observed adverse effect concentration (NOAEC) is 0.5 mg ai/kg (measured 0.47 mg ai/kg). Although this study is classified as supplemental, it is considered scientifically sound and may be used quantitatively in risk assessment. Its supplemental (quantitative) classification stems from not providing food provisions equally across the course of the study (and among colonies) and verification of dietary concentrations only once during the exposure phase of the study.

Reviewer's Consideration of Study Strengths, Limitations and Interpretation

The following strengths and limitations are noted for this study in the context of assessing colony-level risks of oral sulfoxaflor exposures to honey bees.

Strengths:

- Measurement of multiple, colony-level effects which facilitates more holistic interpretation of the results;
- Measurement of residues in hives and in feeding solutions; and
- Long-term of monitoring of endpoints over time.

Limitations:

- Relatively low number of biological replicates (5) compared to other colony feeding studies results in reduced statistical power and greater influence of a single hive on overall results;
- Duration was 10 days, which appears appropriate for evaluating single applications, but may bees might be exposed for longer periods of time with multiple applications during bloom;
- Potential variability with respect to geographic location was not included since all hives were located at a single site;
- Hives were non-randomly placed at the study site, which could introduce bias in the results;
- Food provisions not provided equally to all hives on DAF 100;
- Measurement of sulfoxaflor residues in feeding solutions was done only once during the study, and,
- Storage and transit stability of residue samples were not determined.

9. **ADEQUACY OF THE STUDY:** This study **is considered scientifically sound** and is classified as **supplemental (quantitative)**.

10. **GUIDELINE DEVIATIONS/GLP:** This study was conducted in accordance with Good Laboratory Practices (GLP) Standards:
    - Organisation for Economic Co-operation and Development (OECD) ENV /MC/CHEM (98) 17, 21 January 1998
    - European Community (EC) Commission Directive 2004/10/EC, 11 February 2004 • German Federal Republic Chemicals Act, Annex I, BGBI. J, p. 1146, 11 July 2008

    which are consistent with:
    - Environmental Protection Agency (EPA-FIFRA), Title 40 of the US Code of Federal Regulations Part t 60, 16 October 1989

    This was a non-Guideline, semi-field study (i.e., no OCSPP guidelines have been established) which followed OEPP/EPPO Bulletin No. 22 (Oomen et al., 1992) and OECD guidance document No. 75 (2007)

11. SUBMISSION PURPOSE: Determine the potential effects of GF-2626 (ai: Sulfoxaflor) and its residues on the mortality, colony size, colony performance, overwintering, and brood development of the honeybee (*Apis mellifera* L.) applied via 50% sucrose solution over a 10-day period in a feeding field study in Germany. This study was submitted for the purpose of PRIA (Pesticide Registration Improvement Act).

12. MATERIALS AND METHODS

Test Material:

Identity:	GF-2626 (ai: Sulfoxaflor)
IUPAC name (ai):	Not specified
CAS name (ai):	Not specified
CAS No.:	946578-00-3
Lot No.:	200602464-9
Description:	Liquid / off-white to tan
Purity:	125 g ai/L; 11.8% (analyzed)
Storage:	Ambient (≤30°C), dark, dry

Test Organisms/Hives: The honeybee (*Apis mellifera* L.) was the test organism for this study. Forty-two normally developed, healthy, and queen-right bee colonies with one body including 10 combs were used. Colonies were as homogenous as possible (containing 7670 to 9945 adult bees/colony at study initiation) and originated from one breeding line of sister queens reared at a test facility in 2015. Bees were checked for *Nosema* or *Varroa* disease symptoms, colonies had at least 4-6 brood combs with all brood stages, and at least 1 honey and pollen comb per colony. Colonies were placed 33 days before the start of feeding.

CCA's were initiated on May 25, 2016 (4DBF). The final CCA occurred 302DAF after the end of overwintering.

Test Design: The feeding study test site was located near Pforzheim in Baden-Württemberg, Germany and the hives were in an area with access to natural nectar and pollen stores.. Colony condition was assessed two times prior to exposure, once directly after exposure, eleven times before overwintering, and once after overwintering. For biological assessments, six replicates (one for sampling, i.e. T1s) were exposed to the treated diet for ten days at each treatment level. There were 6 replicates (including one for residue sampling) in the control group that were provided with untreated sucrose solution. Hives were arranged non-randomly in two rows from East to West as illustrated in **Figure 1**.

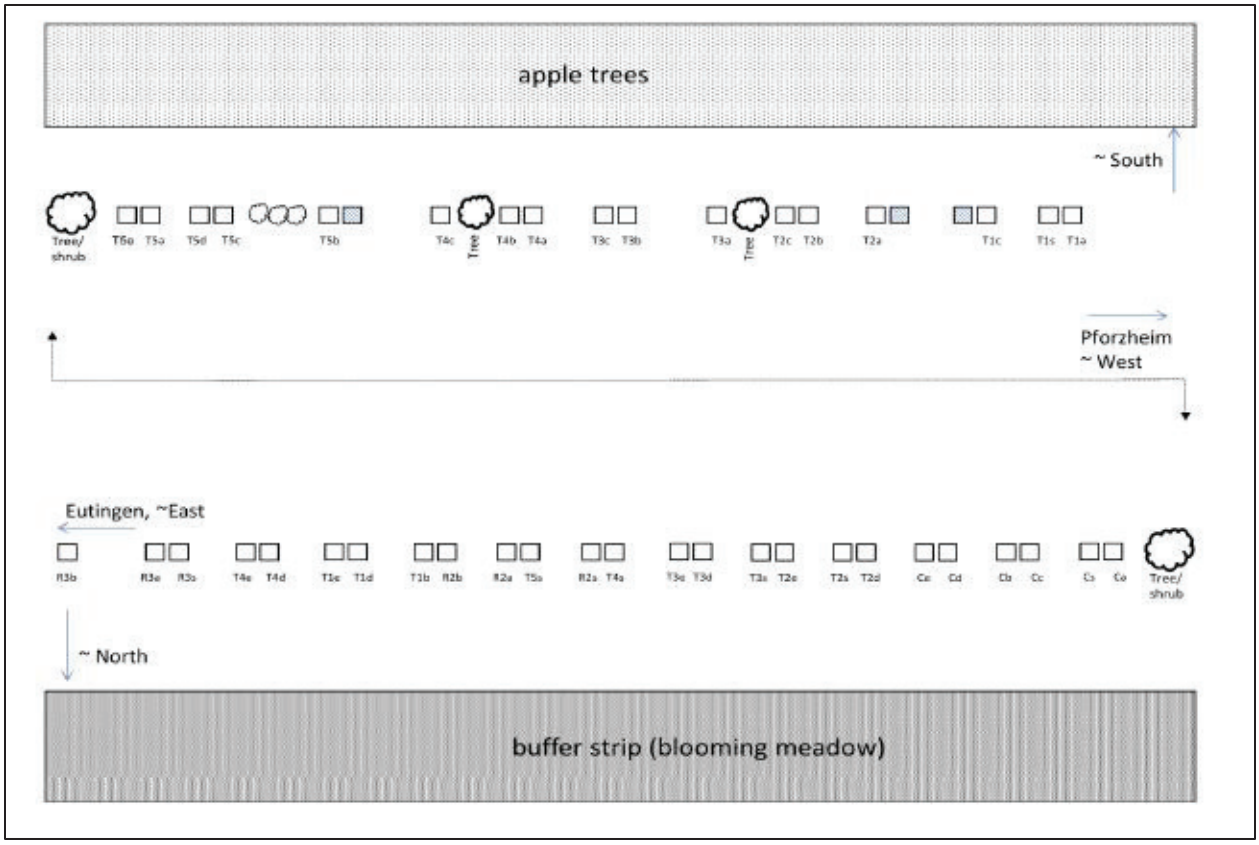


Figure 1. Diagram of the sulfoxaflor colony feeding study site showing locations of honey bee (*Apis mellifera*) hives

Nominal application rates/volumes: Feeding solution target concentrations were 0 (tap water control), 0.02, 0.10, 0.50, 2.0, and 4.0 mg ai/kg in sucrose solution. Two reference materials were tested: Perfekthion (ai: Dimethoate) and Insegar WG (ai: Fenoxycarb).

Feeding (Application): The feeding (application) period was initiated on May 29, 2016. The 50% (w/v) sucrose solution was prepared before feeding by diluting sugar (Südzucker AD) in tap water. Applications of sulfoxaflor or reference material-spiked sucrose solutions or pure 50% sucrose solution (controls) were offered by placing a feeding container inside each colony. Fresh treated sugar syrup was prepared daily for ten days at a feeding volume of 200 mL sucrose/day/colony. Fresh feeding solutions were provided each day. When adding new sugar solution to the hives, the previous feeding's syrup was removed from the feeder and determined to the nearest 1 gram.

Observations: Climatic data were recorded by a data logger and a rain gauge placed on the test site for the entire pre-exposure and exposure period until the start of overwintering. Climatic data from start until end of overwintering were provided by the EAS weather stations "Niefern" and "Enzberg". Air temperature and relative humidity were recorded as daily minimum/maximum/mean whereas precipitation was recorded as a daily sum. Temperature and humidity in boxes used for comb transport and inside the sheltering tent for photographs were also recorded.

Mortality of honey bees was assessed by counting deceased bees in dead bee traps in the front of hives and on the bottom drawer inside hives. Assessments were carried out in replicates intended for biological observations (a-e), and no assessments were carried out in replicates intended for sampling only (Cs, T1s, T2s, T3s, T4s, T5s, R2s, and R3s). Dead bees were differentiated into adult worker bees, pupae, larvae, male bees, and male brood. Mortality was assessed once a day from 4DBF to 1DBF, once shortly before exposure (0DAF), and once a day from 1DAF to 44DAF.

Behavior of honey bees was assessed during the assessments for mortality. The following behaviors were possible: intensive cleaning, trembling, cramping, locomotion problems, inactive bees, filtering bees, and clustering at the hive entrance. Assessments were made in replicates a-d for treatments 1-5 and the control.

Colony condition assessments were conducted twice before application (4DBF and 1DBF), 12 times during exposure and during further monitoring, and once after overwintering (302DAF), totaling 15 assessments. Colonies for residue samplings (Cs, T1s, T2s, T3s, T4s, T5s, R2s, and R3s) were assessed once before application (4DBF). Beekeeper checks were done on each colony assessment day thereafter. The following parameters were assessed: colony strength, presence of a healthy queen, pollen storage area and area with nectar or honey, and area containing cells with eggs, larvae, and capped cells. Comb area containing bees and cells with nectar, pollen, eggs, larvae, and capped cells was estimated per comb side during each CCA. The total number of bees and cells containing single brood stages, pollen, and nectar was also calculated for each colony. During each CCA, colonies were also assessed for bee diseases according to standard beekeeping practice and any unusual occurrences or pests were noted.

For the calculation of the area containing brood and food stages, the following assumptions were made: a comb size of 800 cm^2 per comb side and 400 cells per 100 cm^2. For colony strength, full coverage was assumed to be 130 bees per 100 cm^2.
Bee brood development was assessed in individually marked brood cells over two independent brood cycles. One or several brood combs were taken out of each colony to mark areas containing at least 200 each of eggs, young larvae, and old larvae on the comb on 1DBF (BFD0) and the assessment on 15DAF (BFD0 of second cycle).

The selected combs were uniquely identified. Fixed brood areas were photographed during each brood assessment stage, and digital photos transferred to a computer for further analysis using Hive Analyzer®. The exact positions of the markers on the wooden frames and of each cell were defined in the digital image. Photographic assessments were not conducted during adverse weather conditions.

Honey bee pupae were collected from the hive combs intended for biological evaluations (replicates a-e) once during each brood cycle for weighing and determination of abnormalities. Pupae were collected out of capped cells of brood combs on 19DAF, 20DAF, 32DAF, 33DAF, and 37DAF. All pupae were weighed and checked for abnormalities on the same day and disposed of after completion of the assessment.

Hive weights were recorded continuously once per day, at night to determine the weight development of each colony. Weights were recorded from study start (4DBF) to autumn 2016 (136DAF) and spring 2017 (299DAF, end of overwintering). Hive weights from colony Ca were not recorded due to a malfunction of the hive scale, and no weights were recorded during the overwintering period.

In accordance with local beekeeping practices, two treatments against *Varroa* mites were carried out on July 22, 2016 (54DAF) and August 22, 2016 (85DAF). Treatments were conducted by evaporating formic acid in the hives. For evaluation of the infestation level with Varroa mites, falling mites were counted at each hive used for biological assessments once before overwintering. A *Varroa* board covered with an oil wipe was placed 7 days before counting. Dead and alive mites were counted directly from the board.

Sampling: Whole larvae and pupae were sampled from Cs, T1-5s, R2s, and R3s once before (2DBF) and three times after the start of feeding (11DAF, 19DAF, 45/46DAF). Pre-pupal worker larvae (5^{th} instar) and worker pupae (from capped brood cells) were pulled out of cells with a small spatula.

Honey, nectar, and pollen (bee bread) were sampled from Cs, T1-5s, R2s, and R3s once before and three times after start of feeding – identical to whole larvae/pupae. Pollen was collected using a pollen extractor while nectar and honey were collected using syringes.

Worker jelly was sampled from Cs, T1-5s, R2s, and R3s once before and three times after the start of feeding. Jelly was sampled by removing larvae out of the cells and collecting remaining larval food using a small spatula.

Dose verification was performed once during feeding on 3DAF. Pre-feeding samples were collected from the original diet used before initiation of feeding.

All larvae and pupae, food stores, worker jelly, and dose verification samples were treated in the same way: Control samples were taken before the treated samples or by different staff, and different equipment was used. Samples were split into two sub-samples A (for residue analysis) and R (retained sample) and kept in separate vials. Samples were kept and transported to the facility in Niefern-Öschelbronn on blue or dry ice before being deep-frozen (≤-18°C) within 7 hours and maintained until the start of analysis. Dose verification samples were kept at room temperature for 25 minutes before storage.

Residue Analysis Method: Residue monitoring was carried out at the analytical laboratories of Eurofins Agroscience Services EcoChem GmbH / Eurofins Agroscience Services Ecotox GmbH to assess concentrations of GF-2626 in nectar, sealed honey, pollen, live larvae, live pupae, worker jelly, and feeding solutions.

Statistical Analysis: Colony condition assessments (CCAs) were conducted twice before

application (4 days before feeding (DBF) and 1 DBF), 12 times during exposure and during further monitoring, and once after over-wintering (302 days after feeding (DAF)). A mixed-effects Dunnett's test was conducted for each of the following endpoints across all CCAs using PROC MIXED in SAS ver. 9.4:

- Number of adult bees
- Number of hive cells with eggs present
- Number of hive cells with larvae present
- Number of hive cells with pupae present
- Number of hive cells representing total brood
- Number of hive cells containing nectar
- Number of hive cells containing pollen

A mixed-effects ANOVA model (Dunnett's test) was used to account for the lack of independence (i.e., autocorrelation) between the repeated measures of each endpoint on each hive at each time point (i.e., CCA). Specifically, a Dunnett's test was employed to make comparisons between the effects of individual colony feeding dose treatments and corresponding negative control treatments at each CCA. Several correlation matrices were tested to identify which one best captured the variation in autocorrelation among endpoint observations. A first-order autoregressive correlation structure with heterogeneity (ARH(1)) was determined to minimize the Akaike's Information Criterion (AIC/AICC) and Schwarz's Bayesian Information Criterion (BIC) model selection measurements for each of the endpoints except for number of cells with eggs. Therefore, for consistency, all endpoints were modeled using the ARH(1) correlation structure. Studentized model residuals were visually inspected for normality using Q-Q plots and homoscedasticity by plotting predicted values against studentized residual values. Further details of the EPA statistical analysis can be found in Appendix A.

Summary of Study Dates:

Table 1. Chronological list of activities [a]:

Timing	Date	Activity
33DBF	26 Apr 2016	Set-up of hives
5DBF	24 May 2016	Start of continuous measurement of hive weights
4DBF	25 May 2016	1st colony assessment
4DBF to1DBF	25 May 2016 – 28 May 2016	Daily assessments of mortality and behavior of honeybees before start of feeding
2DBF	27 May 2016	Sampling of all residue matrices in Cs, T1s, T2s, T3s, T4s, T5s, R2s, R3s (S1)
1DBF (=BFD0, 1st brood cycle)	28 May 2016	1st photographic assessment and 2nd colony assessment
0DAF (=BFD+1) to 9DAF	29 May 2016 – 07 Jun 2016	Feeding of treated and untreated sugar solution (=application), 10 feeding dates
0DAF to 44DAF	29 May 2016 - 12 Jul 2016	Daily assessment of mortality and behavior of honeybees after start of feeding

Timing	Date	Activity
3DAF	01 Jun 2016	Sampling of one dose verification (feeding solution), sample in each treatment (S2). (C, T1, T2, T3, T4, T5, R2, R3)
BFD+5, 1st brood cycle	02 Jun 2016	2nd photographic assessment
BFD+10, 1st brood cycle	07 Jun 2016	3rd photographic assessment
1 day after BFD+10 of 1st brood cycle	08 Jun 2016	3rd colony assessment
11DAF	09 Jun 2016	Sampling of all residue matrices in Cs, T1s, T2s, T3s, T4s, T5s, R2s, R3s (S3)
BFD+16, 1st brood cycle; BFD0, 2nd brood cycle	13 Jun 2016	4th photographic assessment and 4th colony assessment; selection of brood cells for 2nd brood cycle
16DAF	14 Jun 2016	Feeding of the colonies via food combs
19DAF and 20DAF	17 Jun 2016 18 Jun 2016	Determination of weight and assessment of morphological abnormalities of pupae
19DAF	17 Jun 2016	Sampling of all residue matrices in Cs, T1s, T2s, T3s, T4s, T5s, R2s, R3s (S4)
BFD+21, 1st brood cycle; BFD+5, 2nd brood cycle	18 Jun 2016	5th photographic assessment
25DAF	23 Jun 2016	Feeding of the colonies (except hive T5s) with sugar solution (Apiinvert)
BFD+11, 2nd brood cycle	24 Jun 2016	6th photographic assessment
BFD+11 of 2nd brood cycle	24 Jun 2016	5th colony assessment
BFD+16, 2nd brood cycle	29 Jun 2016	7th photographic assessment
32DAF, 33DAF, 37DAF	30 Jun 2016, 01 Jul 2016, 05 Jul 2016	Determination of weight and assessment of morphological abnormalities of pupae
BFD+22, 2nd brood cycle	05 Jul 2016	8th photographic assessment
BFD+22, of 2nd brood cycle	05 Jul 2016	6th colony assessment
44DAF	12 Jul 2016	7th colony assessment and last daily assessment of mortality and behaviour of honeybees
45DAF, 46DAF	13 Jul 2016, 14 Jul 2016	Sampling of all residue matrices in Cs, T1s, T2s, T3s, T4s, R2s, R3s (S5)
50DAF	18 Jul 2016	Feeding of the colonies with sugar solution (Apiinvert)
53DAF	21 Jul 2016	8th colony assessment

Timing	Date	Activity
54DAF	22 Jul 2016	1st treatment against *Varroa* mites
12 days after 8th colony assessment	02 Aug 2016	9th colony assessment
72DAF	09 Aug 2016	Feeding of the colonies with sugar solution (Apiinvert)
16 days after 9th colony assessment	18 Aug 2016	10th colony assessment
85DAF	22 Aug 2016	2nd treatment against *Varroa* mites
15 days after 10th colony assessment	02 Sep 2016	11th colony assessment
100DAF	06 Sep 2016	Feeding of the colonies with sugar solution (Apiinvert)
12 days after 11th colony assessment	14 Sep 2016	12th colony assessment
14 days after 12th colony assessment	28 Sep 2016	13th colony assessment
14 days after 13th colony assessment	12 Oct 2016	14th colony assessment and last measurement of hive weight in 2016
12 days after 14th colony assessment	24 Oct 2016	Counting of *Varroa* mites
184DAF	29 Nov 2016	Treatment with oxalic acid
299DAF	24 Mar 2017	Measurement of hive weight
302DAF	27 Mar 2017	15th colony assessment

[a]Data obtained from Table 8 on page 50 of the study report.

13. REPORTED RESULTS

Weather: Rainfall, relative air humidity (%min/max), and temperature (min/max) were recorded until the end of the second brood cycle (4DBF to 44DAF) and achieved values of 174.5 mm, 44.0/100%, and 8.6/32.3ºC, respectively. From 45DAF to 136DAF, rainfall totaled 46.2 mm, humidity ranged from 32.0% to 100%, and temperature ranged from 2.7 to 33.7ºC. During overwintering (137DAF to 302DAF), rainfall totaled 228 mm, humidity ranged from 0.0 to 100%, and temperature ranged from -12.1 to 23.5ºC. Periods of heavy rainfall occurred on 1DAF, 7DAF, 11DAF, and 27DAF. The lowest temperatures were observed in January 2017 and the highest were in March 2017.

A summary of the biological and chemical results is shown in **Table 2.**

Table 2. Summary of biological and chemical results for honey bee colonies fed sulfoxaflor for 10 days

Study Attribute	Results Summary [1]
Test Substance	GF-2626
Timing/Location	2016-17, Baden-Wurttenberg, Germany
Exposure period & Concentration	10 days continuous feeding • 0, 0.02, 0.10, 0.50, 2.0, and 4.0 mg ai/kg (Nominal) • < DL, 0.018, 0.094, 0.47, 1.85, 3.78 mg ai/kg (Measured) • (90%-95% of nominal)
No. Reps. / Treatment	5 (+1 for residue)
Feeding Timing	200 mL sucrose/day/colony, renewed daily
Colonies	42 colonies (sister queens) with 7670 to 9945 adults, 5-10 brood combs, 3-10 honey combs; established 33 days before test initiation
Sucrose Consumption	55% ↓ in daily mean consumption @ 4 mg ai/kg relative to controls. No significant reduction in consumption @ 0.02 – 2 mg ai/kg treatments.
Residues in Hive Matrices	Dose-dependent increase in most hive matrices at 11 DAF**, steep decline by 19 DAF** (except pupae), concentrations **~ LOQ by 45 DAF**. Peak concentrations in nectar > worker jelly> larvae ~ pupae >> pollen
Residue Spike Recovery	90%-101% among various hive matrices & feeding solution
Adult Bee Mortality	• **Before Feeding:** 21-30 dead bees/d all treatments *(NS)* • **During Feeding: 3X ↑ @ 4 mg ai/kg *(S)*** • **1 Wk. Post Feeding: 4X ↑ @ 4 mg ai/kg** (122 dead bees/d; *NS*); 0.02-2 mg ai/kg = 33-45 dead bees/d, *(NS)* • **2 Wk. Post Feeding: 12X ↑ @ 4 mg ai/kg** (238 dead bees/d; ***S***); **6X ↑@ 2 mg ai/kg** (128 dead bees/d; *NS*); 0.02-0.5 mg ai/kg *(NS)* • **3-5 Wk. Post Feeding:** Mortality rates were similar among treatments *(NS)*
Larval and Pupal Bee Mortality	• **Before Feeding:** similar mortality rates all treatments (0.3-0.8 dead bees/d; *NS*) • **During Feeding: 7X ↑ @ 4 mg ai/kg *(S)*** • **1 Wk. Post Feeding: 40X ↑@ 4 mg ai/kg** (12.7 dead bees/d; ***S***); **22X ↑ @ 2 mg ai/kg** (6.8 dead bees/d; ***S***); 0.02-0.5 mg ai/kg = 0.5-0.6 dead bee/d; *NS*) • **2 Wk. Post Feeding: 275X↑ @ 4 mg ai/kg** (56 dead bees/d; ***S***); **580X ↑ @ 2 mg ai/kg** (157 dead bees/d; ***S***); **13X ↑ @ 0.5 mg ai/kg** (2.6 dead bees/d; *NS*); 0.02-0.1 mg ai/kg = 0.9 dead bees/d *(**S** only at 0.02 mg ai/kg)* • **3-4 Wk. Post Feeding:** 4 mg ai/kg (5.5 dead bees/d; ***NS***); 2 mg ai/kg (2.8 dead bees/d; ***S***) 0.02-0.5 mg ai/kg (0.2-0.9 dead bees/d; ***S** only @ 0.02 mg ai/kg in wk 4*) • **5 Wk. Post Feeding:** similar low loss rates at all treatments (0.1-0.3 dead bees/d; *NS*)
Abnormal Behavior	Relatively high number of behavioral abnormalities @ 2 and 4 mg ai/kg (cramping, locomotion problems, and inactive bees). Abnormalities @ 0.02-0.5 mg ai/kg are similar to controls
Colony Strength (Adults)	• **2 & 4 mg ai/kg:** sustained treatment related reductions in # adults @ 9 CCA 5-11 (34-76%; ***S***) • **0.1 & 0.5 mg ai/kg:** slight/sporadic reduction in # adults @ CCA 5-11 (3-25%; *NS*) • **0.02 mg ai/kg:** significant reductions at CCA 6, 9-11 ***(S)***; poor hive strength in one hive prior to exposure; not considered treatment related

Study Attribute	Results Summary [1]
Brood Strength	• **2 & 4 mg ai/kg:** sustained treatment related reductions in total brood (4 to 8 CCAs; 44%-69%; ***S***); Significant reductions in # eggs, larvae, pupae at multiple CCAs ***(S)*** • **0.02-0.5 mg ai/kg**: slight reductions to slight increases total brood, # eggs, larvae, pupae (usually < 15%; *NS*); Significant reduction at CCA5 @ 0.02 mg ai/kg not considered treatment related
Brood Termination Rate	• **4 mg ai/kg** (1st brood cycle): Significant increase in mean brood termination (30%-50%; ***S***) monitored from eggs. Small (<20%) to no increase when monitored from older life stages. No significant increase *(NS)* in brood termination rate for the second brood cycle. • **0.02-2 mg ai/kg:** No significant increase *(NS)* for 1st or 2nd brood cycles monitored from eggs
Brood Index	• **4 mg ai/kg** (1st brood cycle): Significant decrease in mean brood index ***(S)*** monitored from eggs. No significant decrease in brood index for the second brood cycle monitored from eggs. • **0.02-2 mg ai/kg:** No significant decrease *(NS)* for 1st or 2nd brood cycles monitored from eggs
Brood Compensation Rate	• **4 mg ai/kg** (1st brood cycle): Significant decrease in mean brood index (S) monitored from eggs. • **0.02-2 mg ai/kg:** No significant decrease *(NS)* for 1st or 2nd brood cycles monitored from eggs
Food Stores	• **Pollen:** large reduction at multiple CCAs @ 4 mg ai/kg (70%-100%; ***S***); sporadic and small reductions noted @ 0.1 mg ai/kg, but highly inconsistent concentration response pattern. • **Honey:** 30%-70% reduction @ 2 and 4 mg ai/kg during CCA 6 - CCA 15 (***S*** @ CCA8). Smaller reductions @ 0.02-0.5 mg ai/kg, inconsistent concentration-response relationship *(NS)*
Hive Weight	• **2-4 mg ai/kg:** sustained reductions in hive weight (20-25%; ***S***) • **0.02-0.5 mg ai/kg**: smaller reductions (~0-15%; NS) with inconsistent concentration response relationship
Varroa	• No treatment related effects on infestation indicated; non-standard method of monitoring
Overwintering Success and Condition	• **4 mg ai/kg:** 60% overwintering success (2/5 colonies collapsed); Reduced honey stores ***(S)*** • **0-2 mg ai/kg:** 100% overwintering success; Reduced honey stores @ 2 mg ai/kg ***(S)***; significant reduction in pupae and eggs @ 0.02 mg ai/kg not considered treatment related. No other significant effects on brood or food stores.
Overall NOAEC & LOAEC	• **NOAEC = 0.5 mg ai/kg (0.47 mg ai/kg measured)** • **LOAEC = 2 mg ai/kg (1.85 mg ai/kg measured)**
Study Limitations*	1. Relatively low number of replicates (5), resulting in low statistical power 2. All colonies located at a single site (no site-to-site variability) 3. Inconsistent supplemental feeding on 16 DAF 4. Non-random placement of hives 5. Feeding solutions analyzed only once
Reference Toxicant Effects	**Dimethoate (0.86 mg ai/kg);** - similar brood pattern as controls - no sig diff in # dead bees; -slight transient effects **Fenoxycarb (171 mg ai/kg);**

Study Attribute	Results Summary [1]
	- effect on brood pattern - sustained ↑in # dead bees; -effects on total brood and certain stages

[1] S=significantly different from controls ($p<0.05$), NS= not significantly different from controls ($p>0.05$)

Sucrose Consumption

Colonies were fed a total of 2,000 mL of 50% sucrose solution over the 10-day feeding (exposure) period (*i.e.*, 200 ml/d). Control colonies consumed on average 97% of the sucrose solution each day while colonies receiving 0.02, 0.1, 0.5 and 2 mg ai/kg sulfoxaflor consumed between 90% and 97% of the feeding solution each day and there were no statistically significant differences in the volume of diet consumed between control and sulfoxaflor-treated colonies (**Table 3**). However, colonies fed sulfoxaflor at 4 mg/L diet consumed on average significantly ($p<0.05$) less (43% reduction) of the feeding solution relative to controls.

Table 3. Mean, minimum (Min), and maximum (Max) Consumption (in milliliters per colony per day; mL/hive/day) of sucrose feeding solutions by control and sulfoxaflor exposed honey bee (*Apis mellifera*) colonies during 10-day exposure period.

Treatment (mg ai/kg, nominal)	Mean (mL/hive/day)	Min (mL/hive/day)	Max (ml/hive/day)
Control	194.9	174.7	200
0.02 mg ai/kg	195.3	186.5	200
0.1 mg ai/kg	189.5	160.3	200
0.5 mg ai/kg	180.5	172.1	188.4
2 mg ai/kg	185.9	177.2	199.2
4 mg ai/kg	**86.9***	54	112.2

* significantly reduced relative to controls, $P<0.01$; Mann Whitney test

Residues in Hive Matrices

Single samples of hive matrices (*i.e.*, nectar, pollen, worker jelly) and hive bees (larvae, pupae) were analyzed for sulfoxaflor on -2 (before dosing), 11, 19 and 45 DAF **(Figures 2 and 3**). Although the extent of residue sampling was limited (*i.e.,* no replicates and only 4 sampling events), some distinct temporal patterns emerge in the residue profiles. With the exception of residues in pupae (**Figure 3**), sulfoxaflor residues in the other hive matrices sampled peak on DAF 11 (*i.e.*, one day after the end of exposure phase of the study) and declined by factors of ~ 6 to 8-fold by DAF 19. Sulfoxaflor residues measured in pupae peaked on DAF 19. By DAF 45, sulfoxaflor residues in all matrices sampled declined to levels near or below the limits of quantitation (LOQ). These data suggest that sulfoxaflor persistence in hive matrices is ~ 30 days or less following 10 days continuous exposure. This time period is on the order of a single brood cycle (21 days).

The highest peak residues measured were in hive nectar (up to 1.5 mg ai/kg), followed by worker jelly (up to 0.8 mg ai/kg; **Figure 2**), larvae (0.28 mg ai/kg), and pupae (0.15 - 0.2 mg ai/kg; **Figure 3**), and pollen (0.06 mg ai/kg; **Figure 2**). Except for pupae, the highest residues measured where in colonies treated with 2 mg ai/kg; whereas, for pupae, the highest residues

were detected in colonies treated with 4 mg ai/kg. Peak residue concentrations in hive nectar are approximately 50% of the sulfoxaflor concentration in the sucrose feeding solution which may reflect degradation and/or dilution with uncontaminated nectar sources. Peak concentrations of sulfoxaflor in worker jelly are about 25% of those in the sucrose feeding solution. This further reduction in residue concentrations relative to stored nectar may reflect additional degradation and/or dilution during bees' production of worker jelly.

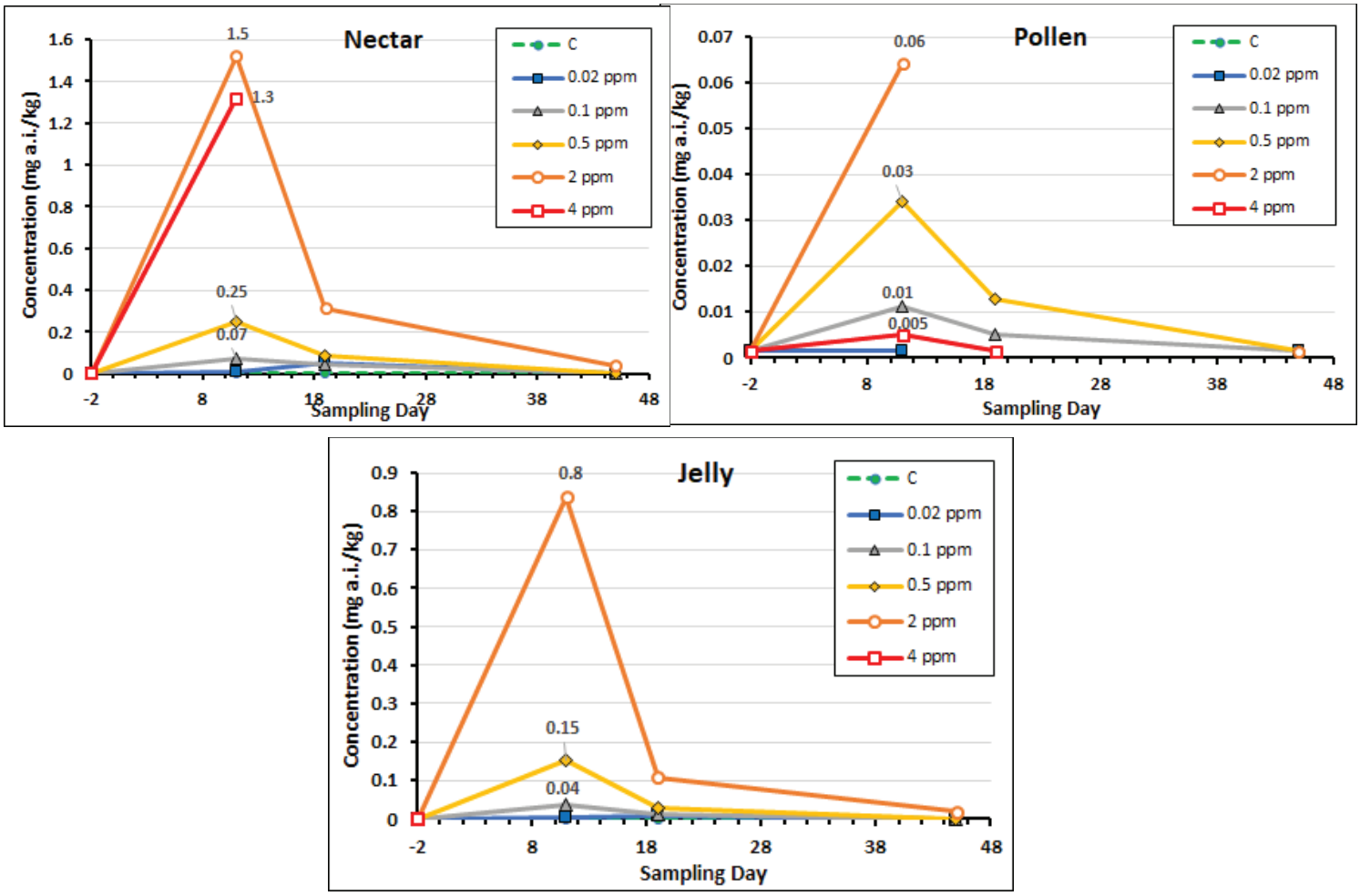


Figure 2. Sulfoxaflor concentrations (in parts per million; = mg ai/kg) measured in nectar, pollen and worker jelly from the monitoring honey bee (*Apis mellifera*) hives from sampling day -2 through 48 days after feeding.

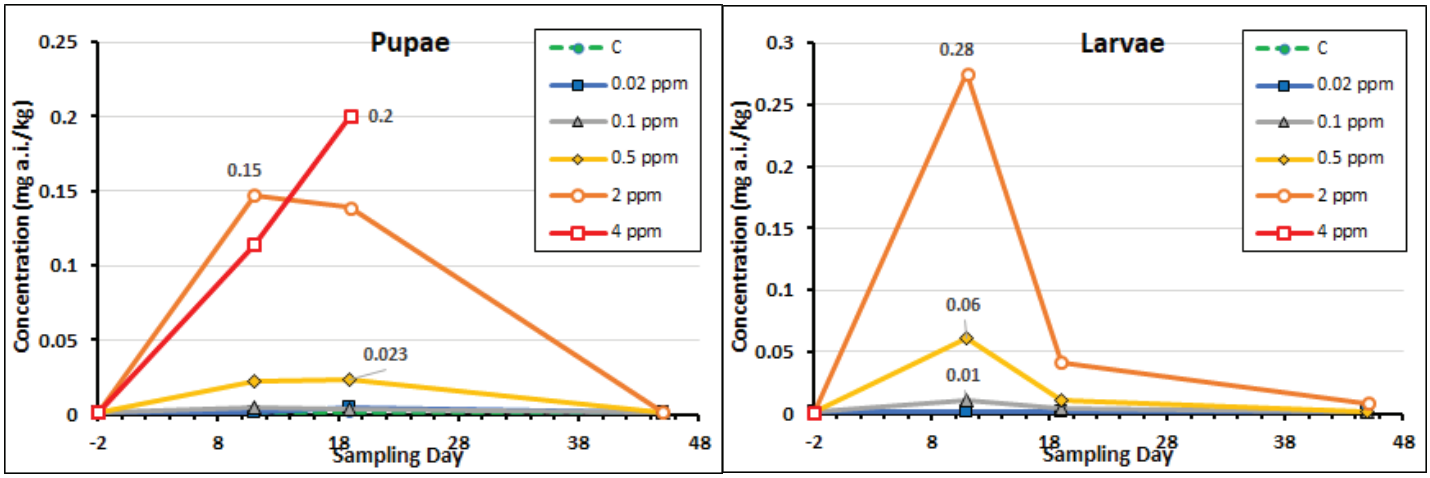


Figure 3. Sulfoxaflor concentrations (in parts per million; ppm; mg ai/kg) measured in honey bee (*Apis mellifera*) larvae and pupae from the monitoring hives from sampling day -2 through 48 days after feeding

Adult and Brood Mortality

Mortality of adult and larval/pupal bees was monitored daily from -4 DAF through 44 DAF during the study. Mortality results, summarized on a weekly basis for adults and brood (i.e., larvae and pupae) are shown in **Tables 4** and **5**, respectively. **Figure 4** depicts daily mean mortality for adults and larvae for each of the study groups. The pattern of mortality measured for adult and immature bees was similar to controls in the lowest three treatments (0.02, 0.1 and 0.5 mg ai/kg; **Figure 4**), with weekly means of adult mortality typically ranging between 15 and 35 bees/day. According to the study authors, the periodic spikes in adult bee mortality observed in these three treatments on Days 12, 17 and 22 did not appear treatment related, as they also occurred in the controls and may reflect low ambient temperatures (*i.e.*, 8-9° C) measured during these days. When summarized on a weekly basis, adult worker mortality was not statistically significant different from controls for the colonies treated with sulfoxaflor at 0.02, 0.1 and 0.5 mg ai/kg. Increased, but not statistically-significant, mortality of adult bees in the 0.5 mg ai/kg treatment on Days 32-33 was due to a single colony (rep C) and was not manifest at 2 and 4 mg ai/kg.

In contrast to the lower three sulfoxaflor treatments (*i.e.,* 0.02, 0.1, and 0.5), adult bee mortality measured in colonies fed sulfoxaflor at 2 mg ai/kg and 4 mg ai/kg increased relative to controls up through 2-weeks post feeding (**Figure 4, Table 4**). For example, statistically-significant ($p<0.05$) increases in mean adult bee mortality (*i.e.*, 49.1 bees/d) during the 10-d feeding period occurred in the 4 mg ai/kg treatment relative to controls (15.4 bees/day). Mean adult bee mortality remained elevated in the 4 mg ai/kg treatment during Week 1 post-feeding (122 bees/day) although it was not statistically significant, and in post-exposure Week 2 (238 bees/day) in which the mortality was significantly ($p<0.05$) different than controls. By Week 3, mean mortality of adults fed 4 mg ai/kg sulfoxaflor was similar (and not significantly different) from controls. Elevated mortality of adult bees fed 2 mg ai/kg sulfoxaflor was evident only during Weeks 1 and 2 post-feeding (44.8 and 128 bees/day) the differences from controls were not statistically significant.

Table 4. Mean (± Standard Deviation) and total mortality of adult honey bees (*Apis mellifera*) recorded before, during and after feeding either untreated (Control) or sulfoxaflor-spiked sucrose solutions for 10 days.

Treatment	Before Feeding			During Feeding			Post Feeding Wk 1			Post-Feeding Wk 2		
	Daily Mean	*SD*	Total	Daily Mean	*SD*	Total	Daily Mean	*SD*	Total	Daily Mean	*SD*	Total
Control	22.7	*18.1*	453	15.4	*11.7*	669	34.6	*28.4*	1211	19.5	*15.8*	684
0.02	21.3	*8.1*	426	12.2	*10.6*	762	32.8	*28.8*	1147	25.1	*20.5*	878
0.10	26.2	*20.5*	524	13.9	*13.9*	815	34.3	*37.6*	1199	20.1	*18.5*	703
0.50	22.5	*14.0*	449	14.8	*10.9*	1168	35.8	*45.1*	1252	21.9	*18.0*	767
2.0	23.8	*12.5*	476	21.2	*40.0*	2699	44.8	*52.6*	1569	128	*89.2*	4468
4.0	29.5	*17.6*	589	**49.1***	*35.0*	669	122	*205*	4269	**238***	*160.6*	8324

Treatment	Post Feeding Wk 3			Post Feeding Wk 4			Post Feeding Wk 5			Table Notes:
	Daily Mean	*SD*	Total	Daily Mean	*SD*	Total	Daily Mean	*SD*	Total	
Control	21.0	*12.1*	734	18.9	*10.3*	660	17.8	*9.2*	534	* = significant ($p<0.05$) increase relative to controls.
0.02	16.9	*11.7*	590	19.2	*10.2*	673	22.8	*11.9*	684	
0.10	18.6	*21.1*	650	14.7	*10.2*	515	14.1	*9.0*	422	
0.5	17.4	*9.6*	608	45.6	*99.2*	1595	14.2	*11.6*	426	Total = total dead bees among the 5 replicate hives during the observation period
2.0	23.9	*26.6*	836	14.8	*7.8*	519	14.4	*13.3*	431	
4.0	29.4	*21.0*	1028	15.7	*10.9*	550	12.4	*10.1*	373	

* = significantly different from controls ($p<0.05$, Wilcox Test)

No statistically-significant difference was detected in mean larvae/pupae mortality in the lower 3 sulfoxaflor treatments (*i.e.*, 0.02, 0.1, and 0.5) relative to controls, except for 0.02 mg ai/kg during Weeks 2 (0.9 bees/day) and 4 (0.5 bees/day) (**Table 5**). These slight but statistically-significant increases in immature bee mortality at 0.02 mg ai/kg are not considered by the study author to biologically significant nor treatment-related. Colonies fed 2 mg ai/kg sulfoxaflor showed statistically-significant increases in immature bee mortality during Weeks 1 through 4 post-feeding, with daily means of 6.8, 157, 2.8 and 1.2 bees/day, in post-exposure Weeks 1, 2, 3 and 4, respectively (**Table 5**). Mean daily mortality in immature bees in post-exposure Week 2 in the 2 mg ai/kg treatment (157 bees/day) was about 3X greater than those in the 4 mg ai/kg treatment (55 bees/day) during the same week.

Table 5. Mean (± Standard Deviation) and total mortality of larval and pupal honey bees (*Apis mellifera*) recorded before, during and after feeding either untreated (Control) or sulfoxaflor-spiked sucrose solutions for 10 days.

Treatment (mg ai/kg)	Before Feeding			During Feeding			Post Feeding Wk 1			Post Feeding Wk 2		
	Daily Mean	*SD*	Total	Daily Mean	*SD*	Total	Daily Mean	*SD*	Total	Daily Mean	*SD*	Total
Control	0.3	0.7	5	0.2	0.5	12	0.3	1.1	12	0.2	0.5	7
0.02	0.7	2.5	13	0.3	0.8	19	0.6	0.9	21	**0.9***	1.2	30
0.10	0.3	0.6	6	0.1	0.4	7	0.5	1.2	19	0.9	1.7	31
0..50	0.5	0.9	9	0.5	1.6	30	0.6	1.1	21	2.6	5.6	92
2.0	0.9	1.4	18	0.8	2.1	43	**6.8***	11.0	237	**157***	265	5488
4.0	0.8	1.1	15	**1.4***	2.1	75	**12.7***	21.9	444	**55.5***	101	1942

Treatment (mg ai/kg)	Post Feeding Wk 3			Post Feeding Wk 4			Post Feeding Wk 5			Table Notes:
	Daily Mean	*SD*	Total	Daily Mean	*SD*	Total	Daily Mean	*SD*	Total	
Control	0.1	0.3	3	0.1	0.2	2	0.3	0.7	9	* = significant ($p<0.05$) increase relative to controls. Total = total dead larvae + pupae among the 5 replicate hives during the observation period
0.02	0.3	0.5	9	**0.5***	0.8	18	0.3	0.6	8	
0.10	0.2	0.6	7	0.3	1.4	12	0.1	0.3	4	
0.50	0.9	2.1	32	0.8	1.9	28	0.2	0.9	6	
2.0	**2.8***	5.1	97	**1.2***	2.4	41	0.1	0.3	3	
4.0	5.5	13.8	191	**1.7***	3.8	61	0.3	0.8	9	

* = significantly different from controls ($p<0.05$, Wilcoxon Test)

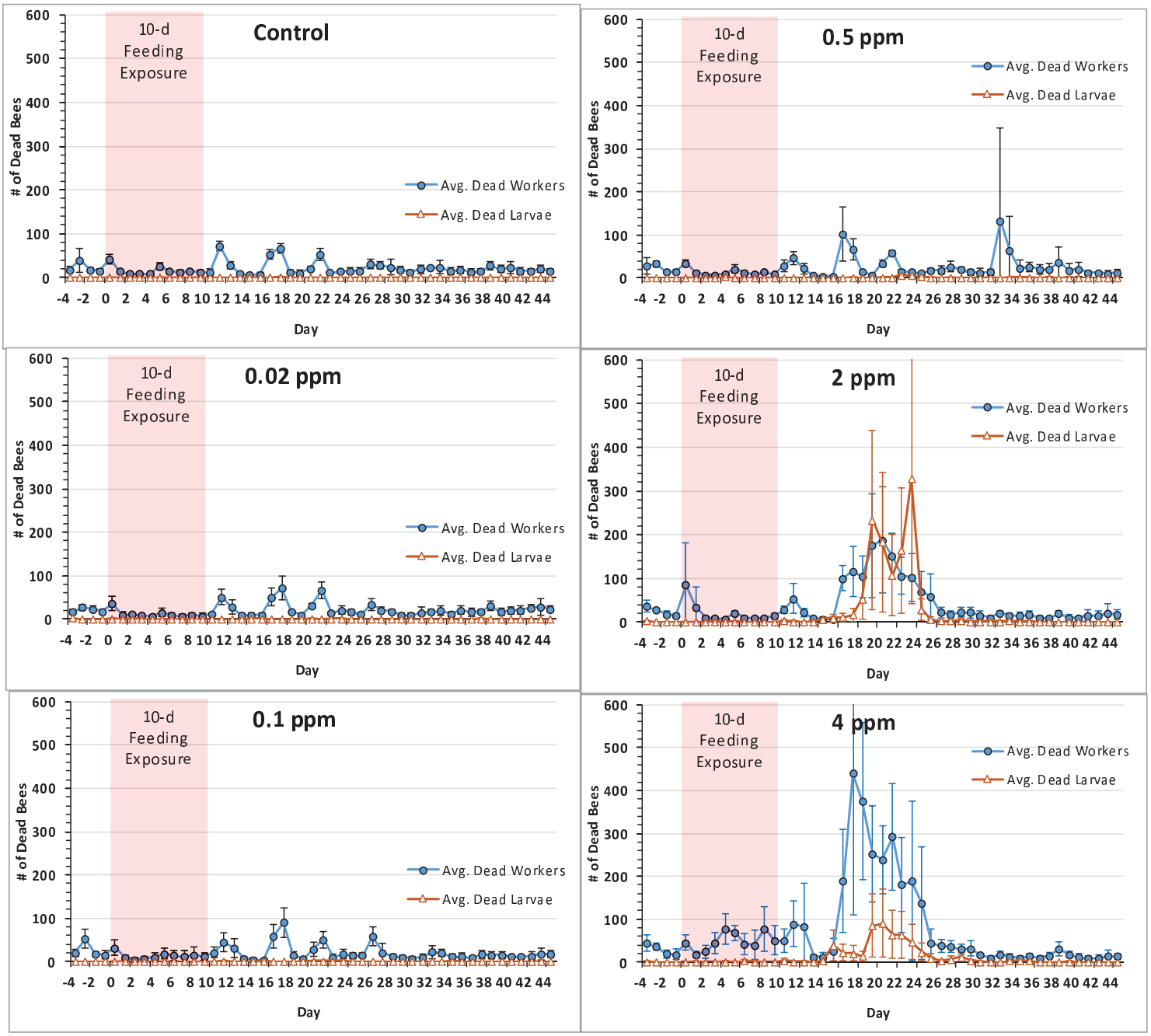
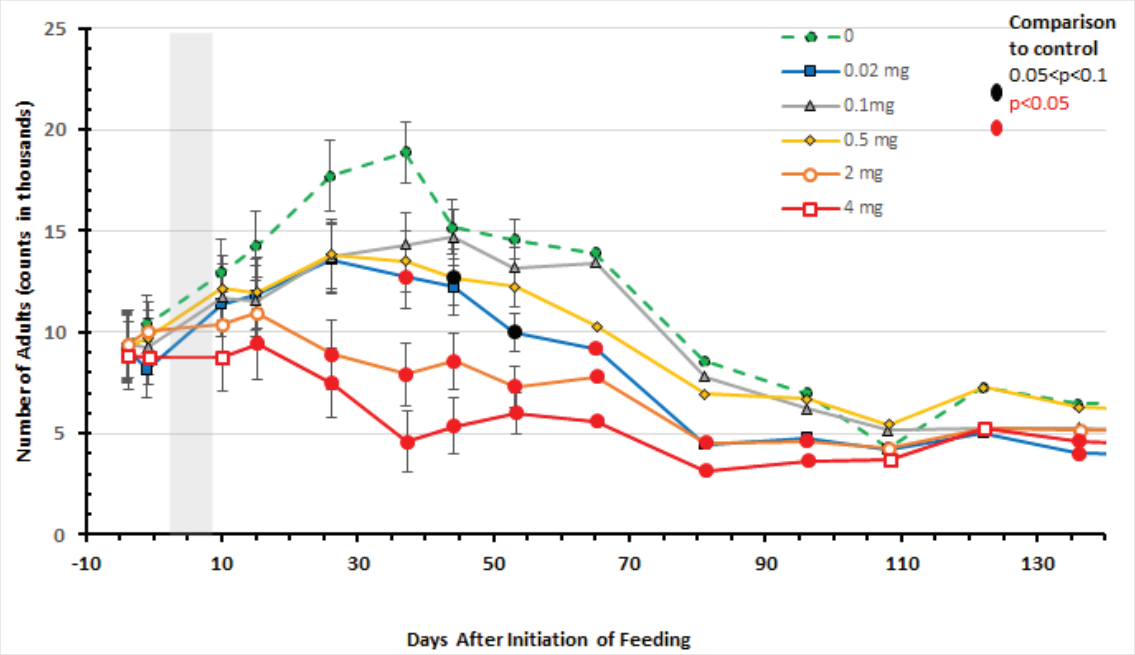


Figure J-4. Mean daily mortality of adult and larval honey bees (*Apis mellifera*) exposed to either control or sulfoxaflor-treated feeding solutions across study days. The 10-day exposure period is highlighted in pink. Error bars reflect 95% confidence limits (ppm=parts per million; mg ai/kg).

Colony Strength and Total Brood

Results from the measurement of colony strength (*i.e.*, total number of adult bees) and total brood in control and sulfoxaflor-treated colonies are shown in **Figure 5.** As depicted in **Figure 5**, colonies fed sulfoxaflor at 2 mg ai/kg or 4 mg ai/kg had statistically significant ($p<0.05$) differences (reductions) relative to controls in the numbers of adult bees and total brood (*i.e.*, eggs, larvae, pupae) following exposure and lasting for most of the monitoring period prior to overwintering. Numbers of adult bees fed 2 and 4 mg ai/kg did not display a spring build up (increase) like control colonies and those colonies exposed to sulfoxaflor at 0.02-0.5 mg ai/kg. No statistically-significant differences in total brood were observed in colonies fed sulfoxaflor at 0.02-0.5 mg ai/kg relative to controls. With the number of adult bees, colonies fed sulfoxaflor at 0.5 mg ai/kg exhibited a difference (reduction) that approached statistically significant ($p <0.1$) relative to controls only at colony condition assessment (CCA) 7, and no statistically-significant reductions were observed in colonies fed 0.1 mg ai/kg sulfoxaflor.

The mean number of adult bees in colonies fed sulfoxaflor at 0.02 mg ai/kg was significantly reduced ($p<0.05$) relative to controls on multiple CCAs following exposure (**Figure 5**, top panel). This finding is unexpected given the general lack of significant differences in adult bees at test concentrations 5X and 25X higher (*i.e.*, 0.1 and 0.5 mg ai/kg).



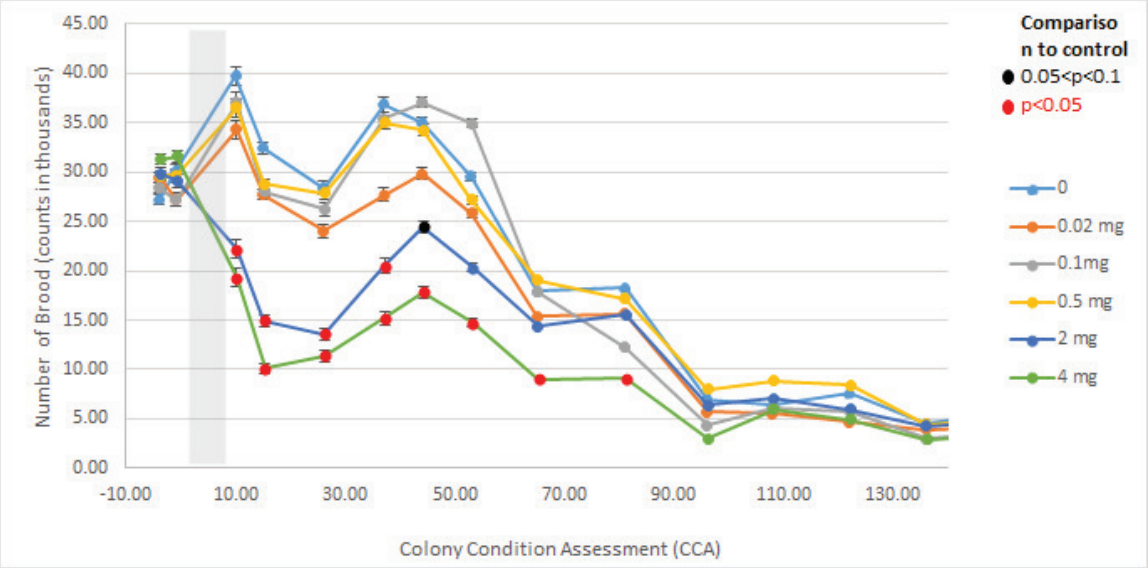


Figure 5. Mean (std. error) of adults (top) and brood (bottom) among sulfoxaflor-treated and control colonies over duration of study. Grey bar reflects the timing of the 10-d feeding period.

According to the study report, data for individual colonies in the 0.02 mg ai/kg treatment indicates that replicate C had less than 50% of adult bees just prior to exposure compared to the other 4 colonies (**Figure 6, bottom panel**). Numbers of adult bees in this colony continued to be low throughout the subsequent 8 CCAs. Furthermore, one colony in the controls (A) contained relatively large numbers of adults throughout the CCAs. With only 5 colonies per treatment, the results from a single colony can have a relatively large impact on statistical results, which may be the case in the comparison of colonies in the 0.02 mg ai/kg treatment to controls.

A second line of evidence is that no biologically or sustained statistically-significant increase in mortality of adult or larval bees occurred in colonies fed 0.02 mg ai/kg sulfoxaflor relative to controls from DAF -4 through DAF 44, as described previously.

A third line of evidence is that food provisions (pollen, nectar) and brood development (described in subsequent sections) were not significantly different from controls in the 0.02 mg ai/kg treatment and were only consistently affected in the 2 and 4 mg ai/kg treatments.

Fourthly, residues measured in hive matrices of colonies fed sulfoxaflor at 0.02 mg ai/kg were 1-2 orders of magnitude below the chronic no-observed effect concentration (NOAEC) for adult bees fed sulfoxaflor in the Tier 1 laboratory test (NOAEC = 0.32 mg ai/kg; LOAEC = 0.58 mg ai/kg). Therefore, direct effects on adult bees fed 0.02 mg ai/kg would not be expected based on the levels of sulfoxaflor measured in the feeding solution or hive matrices.

Finally, colonies fed 0.02 mg ai/kg had levels of *Varroa* mite that were below the commonly accepted threshold of concern (3 mites/100 bees). Therefore, these of evidence suggest that effects on adult numbers observed at 0.02 mg ai/kg are not likely to be treatment related.

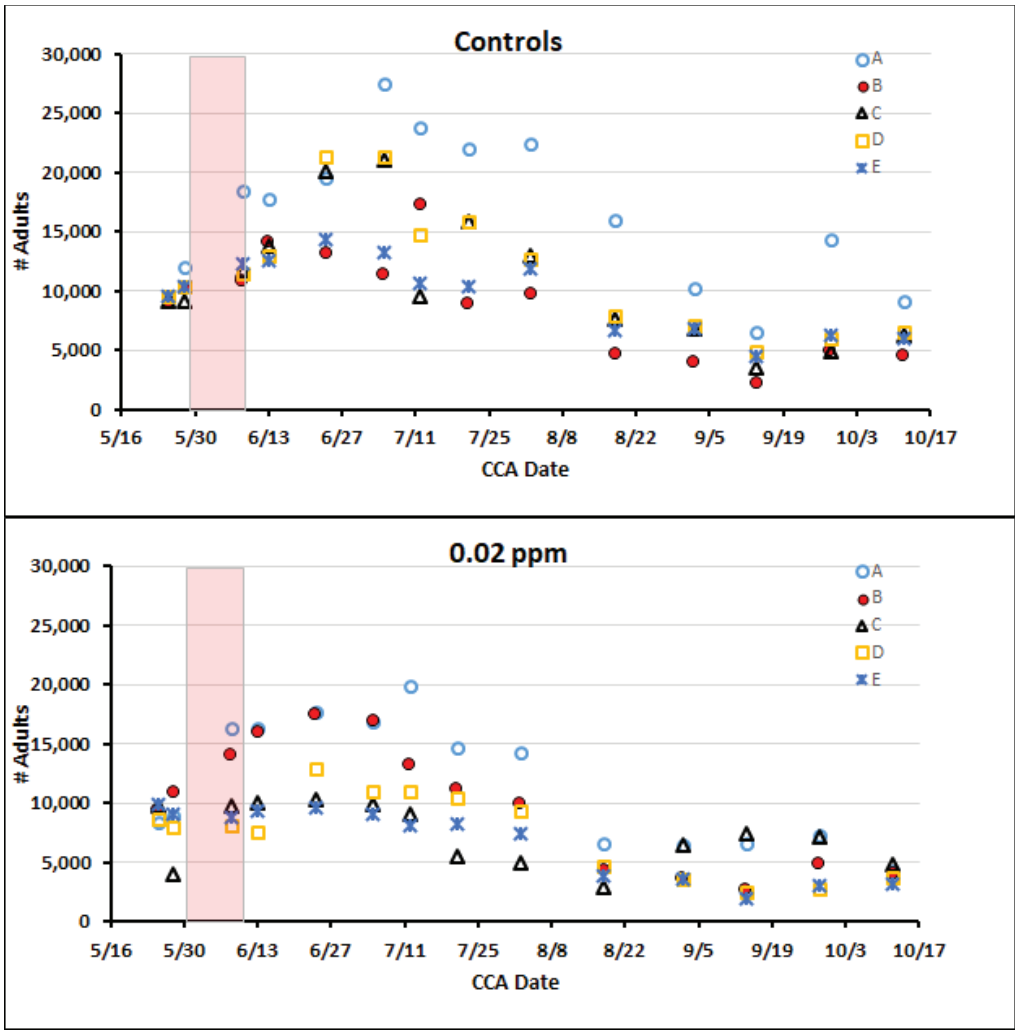


Figure 6. Total numbers of adult honey bees (*Apis mellifera*) from each of the 5 replicate (A – E) control (top) and sulfoxaflor 0.02 mg ai/kg (ppm)-treated (bottom) colonies over the colony condition assessment (CCA dates).

Brood Life Stages

With respect to individual life stages of brood, significant ($p < 0.05$) differences (reductions) were detected in the number of eggs, larvae and pupae in the highest two sulfoxaflor treatments (*i.e.*, 2 and 4 mg ai/kg) relative to controls except for larvae from one CCA in the 0.02 mg ai/kg treatment (**Figure 7**). These findings are consistent with results of overall bee brood mortality described in the preceding section.

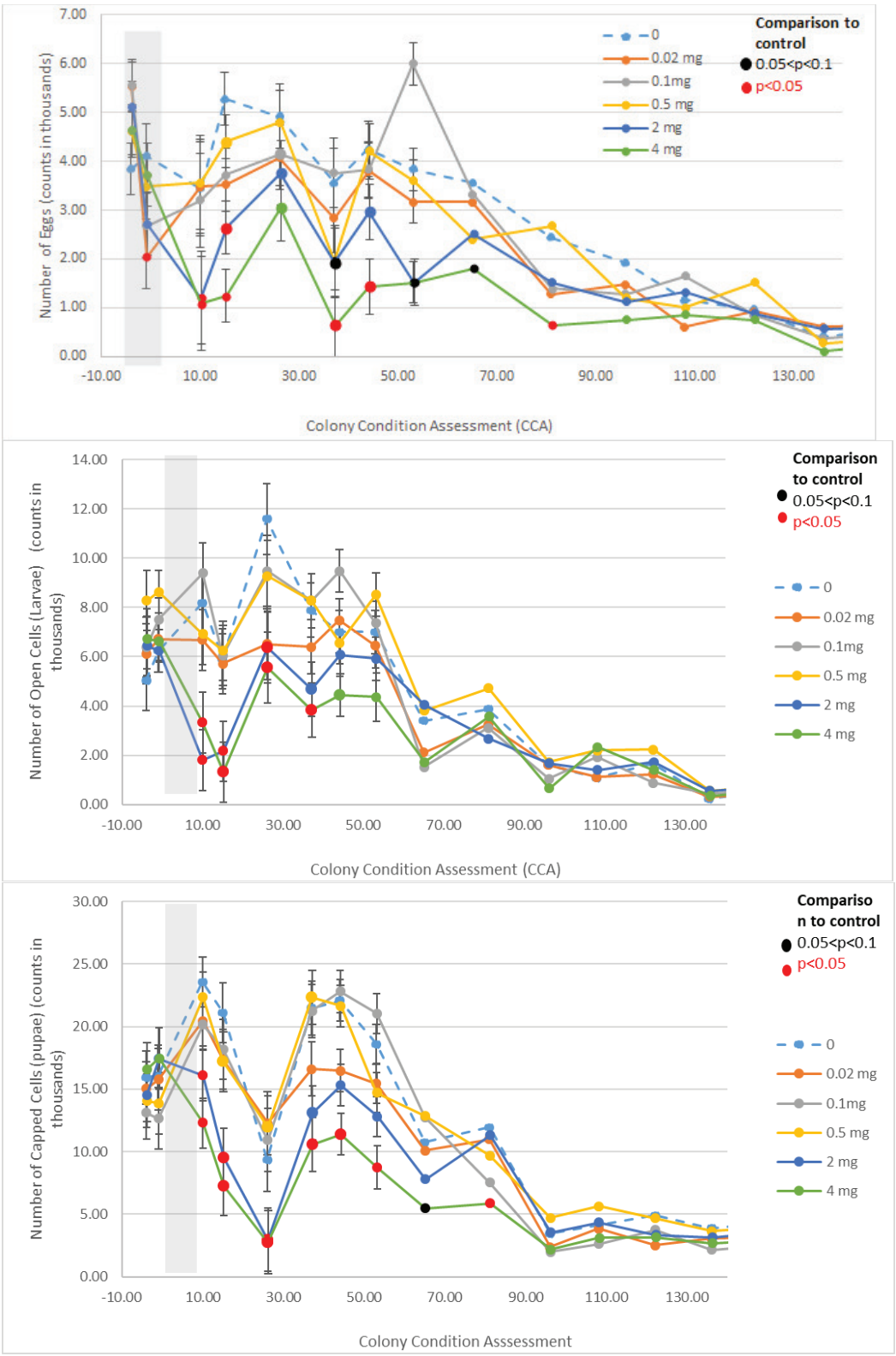


Figure 7. Mean numbers of honey bee (*Apis mellifera*) eggs (top), uncapped cells (larvae; middle) and capped cells (pupae; bottom) in control and sulfoxaflor-treated colonies across colony condition assessments conducted over duration of colony feeding study. Gray bar depicts 10-day exposure phase of the study).

Food Provisions

All colonies (including controls) show an overall decline in the numbers of cells containing pollen during the two CCAs after feeding (**Figure 8**). This decline is then followed by a steady increase in pollen stores over the next 4 CCAs followed by a second gradual decline. The mean number of cells containing pollen was significantly ($p < 0.05$) different (reduced) in hives fed sulfoxaflor at 4 mg ai/kg relative to controls during multiple CCAs. However, beyond this treatment a consistent concentration-response pattern is not indicated. At two CCAs, the number of pollen cells is significantly ($p<0.05$) different (reduced) from controls in hives fed sulfoxaflor at 0.1 mg ai/kg, but not those fed 0.5 mg ai/kg. Pollen provisions in hives fed sulfoxaflor at 2 mg ai/kg were significantly ($p<0.05$) different (reduced) compared to controls only at 1 CCA while no significant differences were detected from controls in hives fed sulfoxaflor at 0.02 and 0.5 mg ai/kg at any CCA.

A gradual increase is seen in the number of cells containing honey following feeding in controls and sulfoxaflor-treated hives over the duration of the CCA measurements. According to the study authors, the "peaks" in honey stores following dosing likely reflected the supplemental feeding during the experiment at 16, 25, 50, 72 and 100 DAF. Statistically significant ($p<0.05$) differences in honey stores relative to controls were only detected at the 2 and 4 mg ai/kg treatments for one CCA.

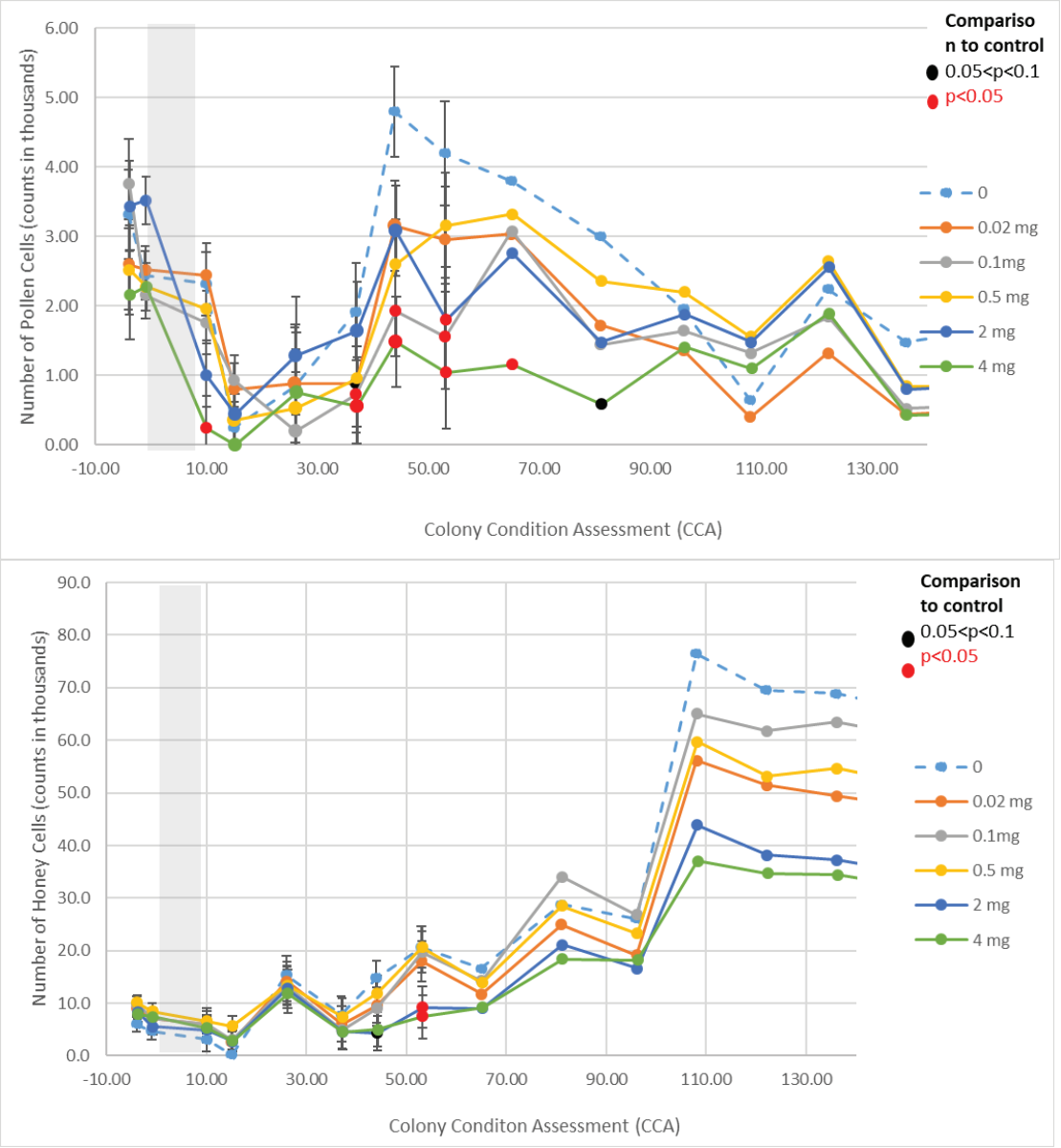


Figure 8. Mean number of cells containing pollen (top panel) and honey (bottom) from control and sulfoxaflor-treated honey bee (*Apis mellifera*) colonies across colony condition assessments conducted over duration of colony feeding study. Gray bar depicts 10-day exposure phase of the study).

Brood Indices

The brood index a measure of the development of brood to the expected life stage and is calculated based on the following ordinal ranking of the life stage present by monitoring a cohort of 200 eggs over a 21-d brood cycle:

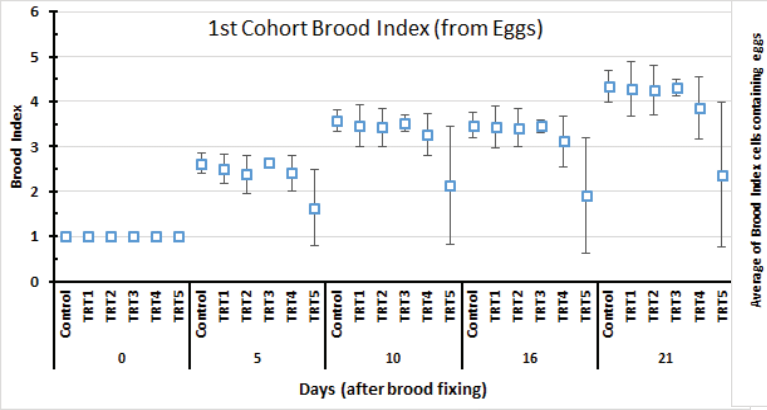
- 0 = empty cell
- 1= egg
- 2= young larvae

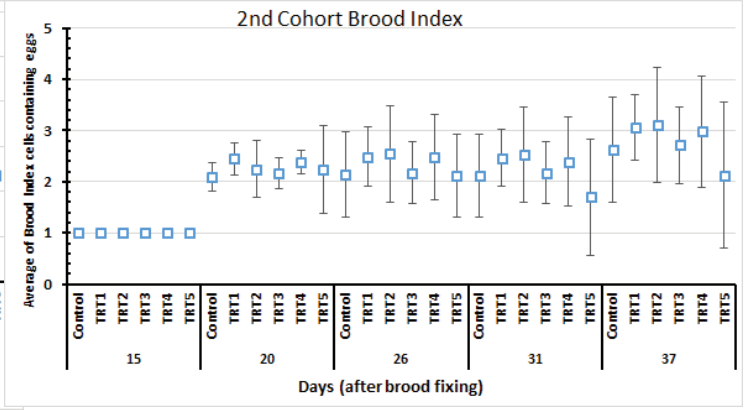
- 3= old larvae
- 4= pupae
- 5= successful hatch

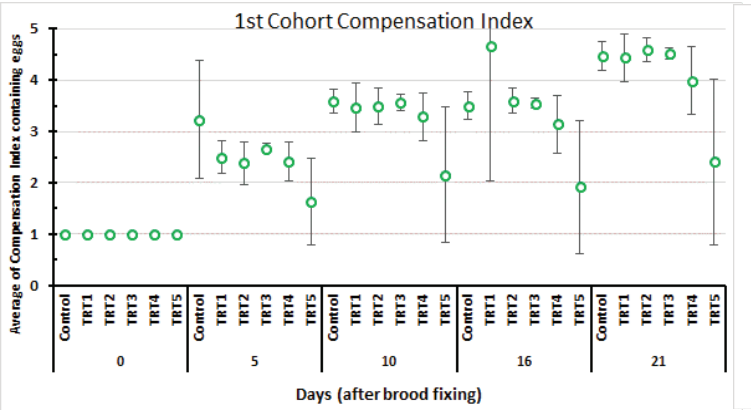
The **Brood Index** is calculated by assigning the above rankings to each cell at selected time intervals over a brood cycle and calculating the average ranking of 200 tracked cells. If the expected brood stage is not present in a cell, it is assigned a "0". The **Brood Compensation Index** is similar to the Brood Index, but if the queen replaces brood in a cell that failed to develop with a new egg, a "1" is assigned to that cell rather than a "0" and its development is tracked and ranked along with the rest of the brood. In this way, the Brood Compensation Index accounts for the ability of the queen to replace brood that fail to develop properly. Consequently, the Brood Compensation Index will be greater than the Brood Index to the extent that the queen replaces failed brood with new eggs and these eggs continue to develop. The **Brood Termination Rate** is simply a measure of the percentage of cells containing brood that did not develop to the expected stage.

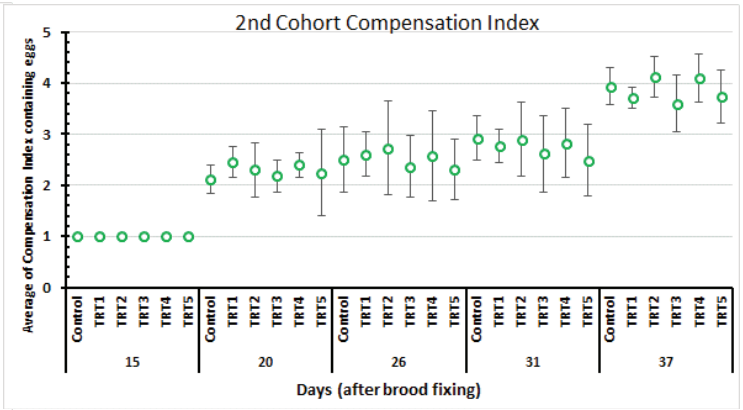
Results from the Brood Index, Brood Compensation Index and Brood Termination Rates of control and sulfoxaflor-treated colonies are summarized in **Figure 9** for brood tracked from the egg stage through pupation among two different brood cycles. The first brood cycle was monitoring from 1 day before feeding (DBF) to 22 days after feeding (DAF). For the first brood cycle, the Brood Index is significantly ($p< 0.05$, Dunnett's test) different (reduced) relative to controls at 5, 10, 16 and 21 DAF in colonies treated with sulfoxaflor at 4 mg ai/kg. Identical results are seen with the Brood Compensation Index (*i.e.*, statistically significant effects only at the highest treatment), except at 16 DAF where no statistically-significant reductions occur. With the Brood Termination Rate, significant ($p<0.05$) differences (increases) from controls increases are seen in the 4 mg ai/kg treatment at 5, 10, 16, and 21 DAF.

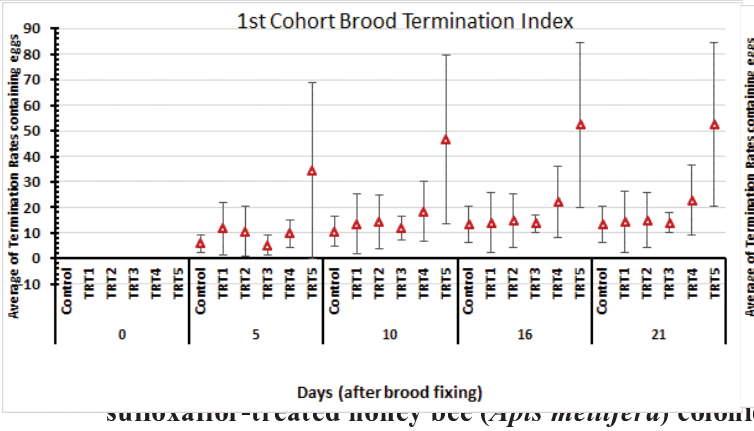
The second brood cycle was monitored from 15 DAF through 37 DAF (22 days). For the second brood cycle, no statistically-significant differences were detected in any sulfoxaflor treatment relative to controls. These data suggest that the impacts on brood development (either direct or indirect) detected in the first brood cycle occurred during and shortly after colonies were fed sulfoxaflor-treated sucrose were transient and did not extend into the second brood cycle.

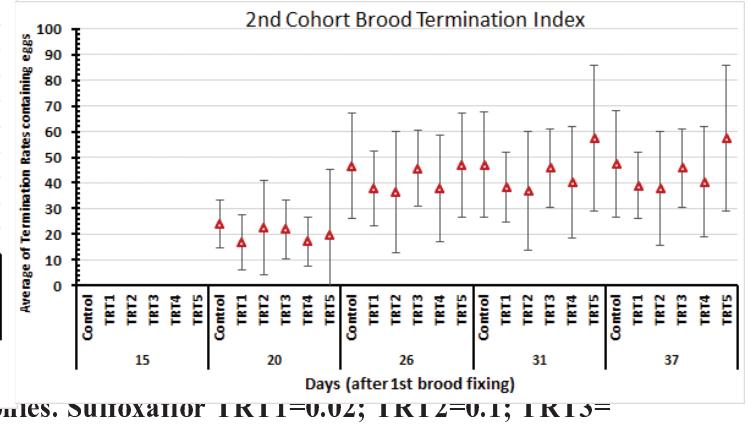












sulfoxaflor-treated honey bee (*Apis mellifera*) colonies. Sulfoxaflor TRT1=0.02; TRT2=0.1; TRT3= 0.5; TRT4=2 and TRT5=4 mg ai/kg.

Hive Weight

The weight of each of the colonies was recorded daily over the duration of the study (except during winter). Results of the mean colony weight for control and sulfoxaflor-treated colonies are depicted in **Figure 10.** Significant ($p < 0.05$) differences (reductions) in weight of colonies treated with sulfoxaflor occurred at 2 and 4 mg ai/kg, relative to controls, shortly after the 10-day dosing period ended (*i.e.*, starting at DAF 22 for the 2 mg ai/kg treatment and at DAF 16 for the 4 mg ai/kg treatment). The colongy weight continued to be significantly different until DAF 66 for colonies traeated with sulfoxaflor at 2 mg ai/kg and until DAF 75 for colonies treated with 4 mg ai/kg with brief reductions shortly thereafter. Beginning near DAF 100, statistically-significant ($p<0.05$) differences (reductions) in hive weight were detected in the 2 and 4 mg ai/kg treatments and continued until DAF 136. A statistically significant ($p<0.05$) differences

(reductions) in hive weight were also detected in the 0.02 mg ai/kg treatment from DAF 133-136; however, for reasons highlighted earlier, this reduciton is not considered likely to be treatment related.

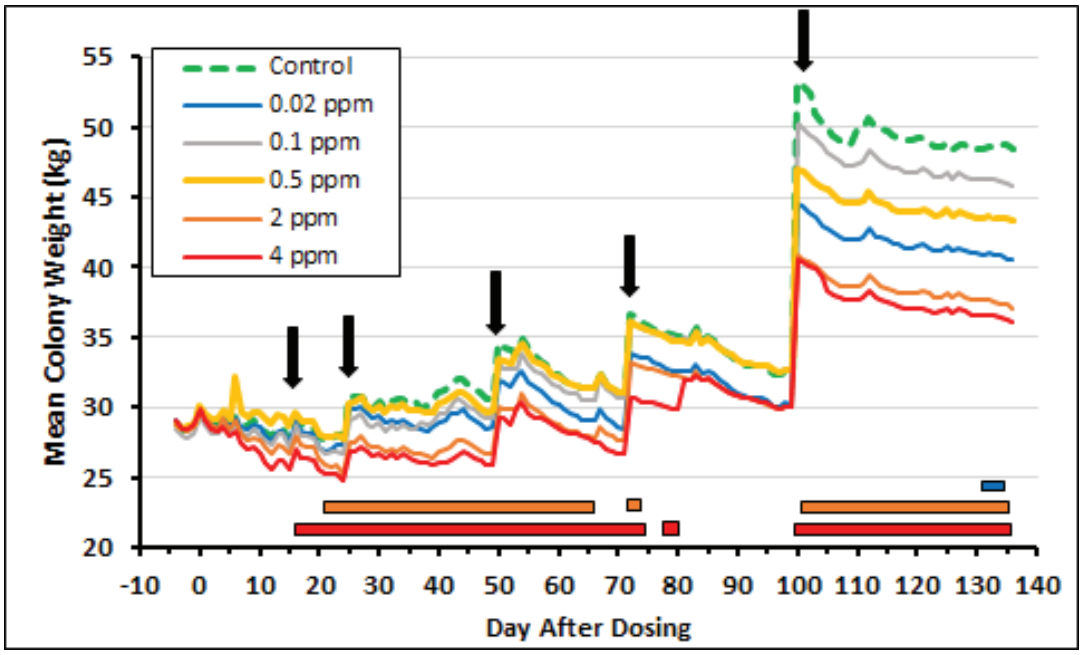


Figure 10. Mean weigh of control and sulfoxaflor-treated honey bee (*Apis mellifera*) colonies. Black arrows indicate days were hives received supplemental feeding. Horizontal bars indicate days in which colony weight was significantly reduced relative to controls (coded according to treatment color; ppm = parts per million equivalent to mg ai/kg).

It is noted here that supplemental feeding of hives on DAF 16 was not uniform among all colonies within sulfoxaflor treatment groups other than contros. Specifically, the study authors report that "food comb" (weight unspecified) was fed to "most colonies" on DAF 16 due to the small amount of food reserves remaining in the hives and lack of flowering plants near the site. Closer inspection of the report indicates that following colonies received this supplemental feeding on DAF 16:

- Controls (all hives)
- 0.02 mg ai/kg (hives b, c, d, e)
- 0.1 mg ai/kg (hives b, c, d, e)
- 0.5 mg ai/kg (hives b, d, e)
- 2 mg ai/kg (hives b, c, d, e)
- 4 mg ai/kg (hives a, c, d, e)

No explanation was provided for this lack of uniformity in hive feeding on DAF 16. Supplemental feeding on the other time periods was uniform across hives within and among treatments.

Varroa

The presence of Varroa mites was monitored once during the fall (October24th) after the exposure period. Hives were monitored by recording the number of mites falling on the bottom of each hive on to sticky traps for seven days. This method is considered a less accurate technique for monitoring the rate of mite infestation of bees compared to other methods (*e.g.,* sampling bees directly via sugar shake method). The number of mites/hive/day recorded for each hive is shown in **Figure 11**. These data indicate no obvious treatment-related effect on infestation by *V. destructor*. Although the overall infestation rate appears low, the methodology used differs from that typically used to measure mite infestation in which the number of mites per 100 bees is determined. Therefore, these results are not necessarily comparable to typical counts of *Varroa* mite infestation.

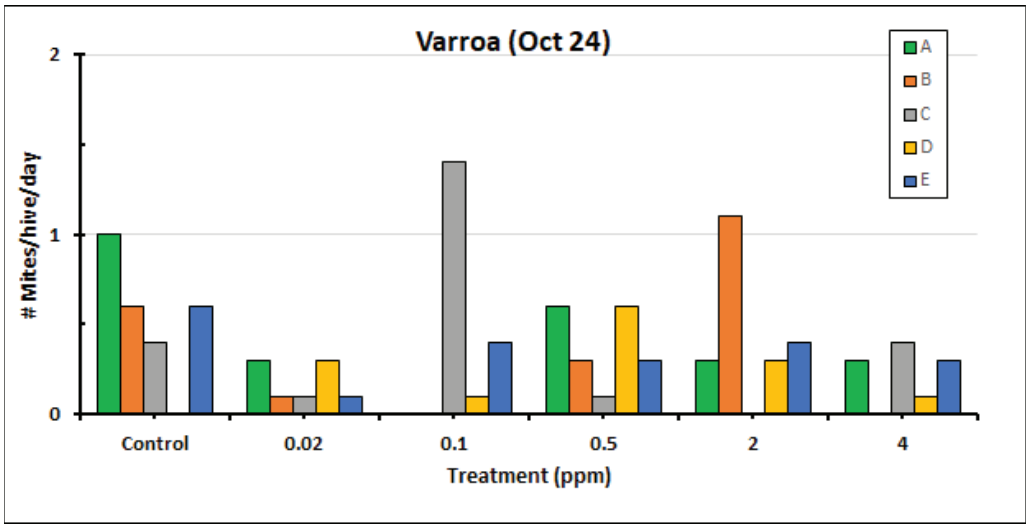


Figure 11. Counts of varroa mites (*Varroa destructor*) in each of the control and sulfoxaflor-treated honey bee colonies in autumn (October 24) prior to overwintering.

Overwintering Success and Condition

All five hives in the control and the sulfoxaflor treatments of 0.02, 0.1, 0.5 and 2 mg ai/kg survived overwintering; whereas, two colonies failed in the 4 mg ai/kg treatment (1 prior to

overwintering at 81 DAF and 1 after overwintering on DAF 299). Statistics were not conducted on overwintering success due to the low number of replicate hives (5).

Measures of colony condition (*i.e.*, overall number of adults, eggs, larvae, pupae, pollen and honey) on the only CCA conducted after overwintering are shown in **Figure 12**. The number of adult bees was significantly ($p<0.05$) different from controls in colonies fed sulfoxaflor at 0.02, 0.1, 0.5, 4 mg ai/kg sulfoxaflor ($p<0.05$) and was approaching statistical significance ($p<0.1$) in colonies fed 2 mg ai/kg sulfoxaflor. However, the study authors considered this measurement as invalid because of the influence of increasing temperatures during the CCA measurement. Specifically, CCAs were conducted in the order of increasing test concentrations (controls first, then 0.02, 0.1, 0.5, 2 and 4 mg ai/kg). During this time, the ambient temperature initially was below 10°C where adult bee foraging would be sporadic (*i.e.*, most of the bees would be in the hive). With subsequent measurements, temperatures increased above 10°C which resulted in more adult bees leaving the hives and actively foraging. Honey bees are known to avoid foraging when temperatures drop below 10°C. Therefore, the lower numbers of adult bees with increasing test concentrations is confounded by the differential foraging activity of bees during their measurement after overwintering.

Statistically significant ($p<0.05$) differences (reduction) in the mean number of eggs and pupae in the colonies were only detected in the 0.02 mg ai/kg treatment (**Figure 12**). Given the complete lack of concentration-response relationship, the study authors did not consider this reduction to be treatment related. No statistically significant differences were detected in the number of cells containing larvae or pollen in any sulfoxaflor treatment relative to controls. However, honey stores were significantly ($p<0.05$) different (reduced) compared to controls for colonies treated with sulfoxaflor at 2 and 4 mg ai/kg (**Figure 12**).

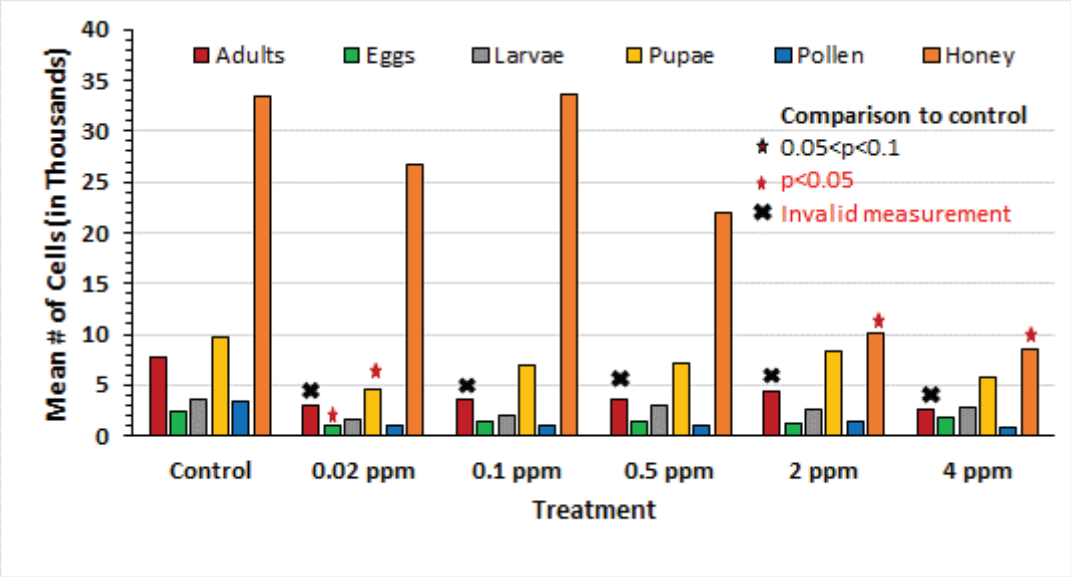


Figure 12. Colony condition assessment of control and sulfoxaflor-treated hives on DAF 299 after overwintering

Mortality: Mean worker bee mortality during the first brood cycle (1DAF to 20AF) was

14. Reviewer's Consideration of Study Strengths, Limitations and Interpretation

The following strengths and limitations are noted for this study in the context of assessing colony-level risks of oral sulfoxaflor exposures to honey bees.

Strengths:

- Measurement of multiple, colony-level effects which facilitates more holistic interpretation of the results;
- Measurement of residues in hives and in feeding solutions; and
- Long-term of monitoring of endpoints over time.

Limitations:

- Relatively low number of biological replicates (5) compared to other colony feeding studies results in reduced statistical power and greater influence of a single hive on overall results;

- Duration was 10 days, which appears appropriate for evaluating single applications, but may bees might be exposed for longer periods of time with multiple applications during bloom;
- Potential variability with respect to geographic location was not included since all hives were located at a single site;
- Hives were non-randomly placed at the study site, which could introduce bias in the results;
- Food provisions not provided equally to all hives on DAF 100;
- Measurement of sulfoxaflor residues in feeding solutions was done only once during the study, and,
- Storage and transit stability of residue samples were not determined.

15. REVIEWER'S COMMENTS

Preliminary non-GLP assessments of hives took place from May 16-20, 2016. Study initiation took place June 6, 2016. The start of the experimental GLP-phase (field) was July 13, 2016. The end of the experimental phase was April 13, 2017. Study completion date was October 19, 2017.

Signed and Dated No Data Confidentiality, GLP, and Quality Assurance statements were provided. This study was conducted in accordance with OECD ENV/MC/CHEM (98) Good Laboratory Practice. Weather data from EAS-weather station and GPS data are not generated under GLP.

16. REFERENCES

OECD guidance document No. 75 (2007): Guidance document on the honey bee (*Apis mellifera L.)* brood test under semi-field conditions. OECD Environment, Health and Safety Publications Series on Testing and Assessment No. 75, ENV/JM/MONO(2007)22.

OOMEN P.A., DE RUIJTER A. & J. VAN DER STEEN (1992): Method for honeybee brood feeding tests with insect growth-regulating insecticides. - EPPO Bulletin 22, 613 - 616.

Appendix A. Details of Statistical Methods and Results